

54-000170PC.ST251
SEQUENCE LISTING

<110> The Scripps Research Institute
Schultz, Peter
Wang, Lei

<120> SITE SPECIFIC INCORPORATION OF KETO AMINO ACIDS INTO PROTEINS

<130> 54A-000170PC

<140> PCT/US 03/32576

<141> 2003-10-15

<160> 24

<170> PatentIn version 3.1

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<212> DNA

<213> artificial

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<223> tRNA synthetase based on M. jannaschii tyrosyl-tRNA synthetase

<400> 1

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ccaattagaa agagatta                                     918

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<210> 3
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<220>
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ggagaaggaa agatgagttc ttcaaaaggg aattttatag ctgttgatga ctctccagaa      660
gagattaggg ctaagataaa gaaagcatac tgcccagctg gagttgttga aggaaatcca      720
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 tttgggtggag atttgacagt taatagctat gaggagttag agagtttatt taaaaataag 840
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 ccaattagaa agagatta 918

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<220>
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 gatataatta tattgttggc tgatttacac gcctatttaa accagaaagg agagttggat 240
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<220>
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gatgaaaatc caaagggttc tgaagttatc tatccaataa tgcagggttaa tacgtatcat 480
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tttgggtggag atttgacagt taatagctat gaggagttag agagtttatt taaaaataag 840
gaattgcac caatggattt aaaaaatgct gtagctgaag aacttataaa gatttttagag 900
ccaattagaa agagatta 918

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<210> 7
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<213> artificial

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<400> 7

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gagattagaa aaataggaga ttataacaaa aaagtttttg aagcaatggg gttaaaggca      300
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ttggcttttaa aaactacctt aaaaagagca agaaggagta tggaacttat agcaagagag      420
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agggagcttt taccaaaaaa gggtgtttgt attcacaacc ctgtcttaac gggtttggat      600
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gagattaggg ctaagataaa gaaagcatat tgcccagctg gagttgttga aggaaatcca      720
ataatggaga tagctaaata cttccttgaa taccctttta ccataaaaag gccagaaaaa      780
tttgggtggag atttgacagt taatagctat gaggagttag agagtttatt taaaaataag      840
gaattgcata caatggattt aaaaaatgct gtagctgaag aacttataaa gattttagag      900
ccaattagaa agagatta                                     918

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<210> 8

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<212> DNA

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atacatttag ggcattatct ccaaataaaa aagatgattg atttacaaaa tgctggattt      180
gatataatta tattgttggc tgatttacac gcctatttaa accagaaagg agagttggat      240
gagattagaa aaataggaga ttataacaaa aaagtttttg aagcaatggg gttaaaggca      300
aaatatgttt atggaagtca tttccagctt gataaggatt atacactgaa tgtctataga      360
ttggcttttaa aaactacctt aaaaagagca agaaggagta tggaacttat agcaagagag      420
gatgaaaatc caaagggtgc tgaagttatc tatccaataa tgcagggtta tcagactcat      480
tatgagggcg ttgatgttgc agttggaggg atggagcaga gaaaaatata catgttagca      540
agggagcttt taccaaaaaa gggtgtttgt attcacaacc ctgtcttaac gggtttggat      600

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ggagaaggaa agatgagttc ttcaaaaggg aattttatag ctgttgatga ctctccagaa 660
gagattaggg ctaagataaa gaaagcatac tgcccagctg gagttggtga aggaaatcca 720
ataatggaga tagctaaata cttccttgaa tatccttttaa ccataaaaag gccagaaaaa 780
tttggtggag atttgacagt taatagctat gaggagttag agagtttatt taaaaataag 840
gaattgcac caatggattt aaaaaatgct gtagctgaag aacttataaa gatttttagag 900
ccaattagaa agagatta 918

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<210> 9
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gagattagaa aaataggaga ttataacaaa aaagtttttg aagcaatggg gttaaaggca 300
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tttggtggag atttgacagt taatagctat gaggagttag agagtttatt taaaaataag 840
gaattgcac caatggattt aaaaaatgct gtagctgaag aacttataaa gatttttagag 900
ccaattagaa agagatta 918

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atacatttag ggcattatct ccaaataaaa aagatgattg atttacaaaa tgctggattt 180
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gagattagaa aaataggaga ttataacaaa aaagtttttg aagcaatggg gttaaaggca 300
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ttggctttta aaactacctt aaaaagagca agaaggagta tggaacttat agcaagagag 420
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ataatggaga tagctaaata cttccttgaa tatectttta ccataaaaag gccagaaaaa 780
tttgggtggag atttgacagt taatagctat gaggagttag agagtttatt taaaaataag 840
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ccaattagaa agagatta 918

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atacatttag ggcattatct ccaaataaaa aagatgattg atttacaaaa tgctggattt 180
gatataatta tattgttggc tgatttacac gcctatttaa accagaaagg agagttggat 240
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tttgggtggag atttgacagt taatagctat gaggagttag agagtttatt taaaaataag 840
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ccaattagaa agagatta

918

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<400> 12

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atacatttag ggcattatct ccaaataaaa aagatgattg atttacaaaa tgctggattt      180
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gagattagaa aaataggaga ttataacaaa aaagtttttg aagcaatggg gttaaaggca      300
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tctagtggcg ttgatgttgc agttggaggg atggagcaga gaaaaataca catgttagca      540
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gagattaggg ctaagataaa gaaagcatat tgcccagctg gagttgttga aggaaatcca      720
ataatggaga tagctaaata cttccttgaa tatcctttaa ccataaaaag gccagaaaaa      780
tttggtggag atttgacagt taatagctat gaggagttag agagtttatt taaaaataag      840
gaattgcata caatggattt aaaaaatgct gtagctgaag aacttataaa gatttttagag      900
ccaattagaa agagatta                                          918

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<400> 13

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atacatttag ggcattatct ccaaataaaa aagatgattg atttacaaaa tgctggattt      180
gatataatta tattgttggc tgatttacac gcctatttaa accagaaagg agagttggat      240
gagattagaa aaataggaga ttataacaaa aaagtttttg aagcaatggg gttaaaggca      300
aaatatgttt atggaagttc cttccagctt gataaggatt atacactgaa tgtctataga      360
ttggctttta aaactacctt aaaaagagca agaaggagta tggaacttat agcaagagag      420

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gatgaaaatc caaagggttc tgaagttatc tatccaataa tgcagggttaa tacgagtcac 480
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 ggagaaggaa agatgagttc ttcaaaaggg aattttatag ctgttgatga ctctccagaa 660
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 atacatttag ggcattatct ccaaataaaa aagatgattg atttataaaa tgctggattt 180
 gatataatta tattgttggc tgatttacac gcctatttaa accagaaagg agagttggat 240
 gagattagaa aaataggaga ttataacaaa aaagtttttg aagcaatggg gttaaaggca 300
 aaatatgttt atggaagtaa tttccagctt gataaggatt atacactgaa tgtctataga 360
 ttggctttta aaactacctt aaaaagagca agaaggagta tggaacttat agcaagagag 420
 gatgaaaatc caaagggttc tgaagttatc tatccaataa tgcagggttaa tccgcttcac 480
 tatcagggcg ttgatgttgc agttggaggg atggagcaga gaaaaatata catgttagca 540
 agggagcttt taccaaaaaa ggttggttgc attcacaacc ctgtcttaac gggtttggat 600
 ggagaaggaa agatgagttc ttcaaaaggg aattttatag ctgttgatga ctctccagaa 660
 gagattaggg ctaagataaa gaaagcatac tgcccagctg gagttggtga aggaaatcca 720
 ataattggaga tagctaaata cttccttgaa tatcctttta ccataaaaaag gccagaaaaa 780
 tttggtggag atttgacagt taatagctat gaggagttag agagtttatt taaaaataag 840
 gaattgcac caatggattt aaaaaatgct gtagctgaag aacttataaa gatttttagag 900
 ccaattagaa agagatta 918

<210> 15
 <211> 918
 <212> DNA
 <213> artificial

<220>
 <223> tRNA synthetase based on M. jannaschii tyrosyl-tRNA synthetase

<400> 15
 atggacgaat ttgaaatgat aaagagaaac acatctgaaa ttatcagcga ggaagagtta 60
 agagaggttt taaaaaaaga tgaaaaatct gctacgatag gttttgaacc aagtggtaaa 120
 atacatttag ggcattatct ccaaataaaa aagatgattg atttacaaaa tgctggattt 180
 gatataatta tattgttggc tgatttacac gcctatttaa accagaaagg agagttggat 240
 gagattagaa aaataggaga ttataacaaa aaagtttttg aagcaatggg gttaaaggca 300
 aaatatgttt atggaagtct gttccagctt gataaggatt atacactgaa tgtctataga 360
 ttggctttta aaactacctt aaaaagagca agaaggagta tggaaacttat agcaagagag 420
 gatgaaaatc caaaggttgc tgaagttatc tatccaataa tgcagggtta tcctcttcat 480
 tatgagggcg ttgatgttgc agttggaggg atggagcaga gaaaaataca catgttagca 540
 agggagcttt taccaaaaaa ggttgtttgt attcacaacc ctgtcttaac gggtttggat 600
 ggagaaggaa agatgagttc ttcaaaaggg aattttatag ctgttgatga ctctccagaa 660
 gagattaggg ctaagataaa gaaagcatac tgcccagctg gagttgttga aggaaatcca 720
 ataatggaga tagctaaata cttccttgaa tctcctttta ccataaaaaag gccagaaaaa 780
 tttggtggag atttgacagt taatagctat gaggagttag agagtttatt taaaaataag 840
 gaattgcac caatggattt aaaaaatgct gtagctgaag aacttataaa gatttttagag 900
 ccaattagaa agagatta 918

<210> 16
 <211> 918
 <212> DNA
 <213> artificial

<220>
 <223> tRNA synthetase based on M. jannaschii tyrosyl-tRNA synthetase

<400> 16
 atggacgaat ttgaaatgat aaagagaaac acatctgaaa ttatcagcga ggaagagtta 60
 agagaggttt taaaaaaaga tgaaaaatct gctcttatag gttttgaacc aagtggtaaa 120
 atacatttag ggcattatct ccaaataaaa aagatgattg atttacaaaa tgctggattt 180
 gatataatta tattgttggc tgatttacac gcctatttaa accagaaagg agagttggat 240
 gagattagaa aaataggaga ttataacaaa aaagtttttg aagcaatggg gttaaaggca 300
 aaatatgttt atggaagtac tttccagctt gataaggatt atacactgaa tgtctataga 360
 ttggctttta aaactacctt aaaaagagca agaaggagta tggaaacttat agcaagagag 420
 gatgaaaatc caaaggttgc tgaagttatc tatccaataa tgcagggtta tccggttcat 480
 tatcagggcg ttgatgttgc agttggaggg atggagcaga gaaaaataca catgttagca 540
 agggagcttt taccaaaaaa ggttgtttgt attcacaacc ctgtcttaac gggtttggat 600
 ggagaaggaa agatgagttc ttcaaaaggg aattttatag ctgttgatga ctctccagaa 660
 gagattaggg ctaagataaa gaaagcatac tgcccagctg gagttgttga aggaaatcca 720

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ataatggaga tagctaaata cttccttgaa tacccttttaa ccataaaaag gccagaaaaa 780
 tttggtggag atttgacagt taatagctat gaggagttag agagtttatt taaaaataag 840
 gaattgcac caatggattt aaaaaatgct gtagctgaag aacttataaa gatttttagag 900
 ccaattagaa agagatta 918

<210> 17
 <211> 918
 <212> DNA
 <213> artificial

<220>
 <223> tRNA synthetase based on M. jannaschii tyrosyl-tRNA synthetase

<400> 17
 atggacgaat ttgaaatgat aaagagaaac acatctgaaa ttatcagcga ggaagagtta 60
 agagaggttt taaaaaaaga tgaaaaatct gctactatag gttttgaacc aagtggtaaa 120
 atacatttag ggcattatct ccaaataaaa aagatgattg atttataaaa tgctggattt 180
 gatataatta tattgtttggc tgatttacac gcctatttaa accagaaagg agagttggat 240
 gagattagaa aaataggaga ttataacaaa aaagtttttg aagcaatggg gttaaaggca 300
 aaatatgttt atggaagtgc gttccagctt gataaggatt atacactgaa tgtctataga 360
 ttggctttta aaactacctt aaaaagagca agaaggagta tggaacttat agcaagagag 420
 gatgaaaatc caaagggttc tgaagttatc tatccaataa tgcagggtta tccactgcat 480
 tatcagggcg ttgatgttgc agttggaggg atggagcaga gaaaaataca catgttagca 540
 agggagcttt taccaaaaaa ggttgtttgt attcacaacc ctgtcttaac gggtttggat 600
 ggagaaggaa agatgagttc ttcaaaaggg aattttatag ctgttgatga ctctccagaa 660
 gagattaggg ctaagataaa gaaagcatac tgcccagctg gagttgttga aggaaatcca 720
 ataatggaga tagctaaata cttccttgaa tacccttttaa ccataaaaag gccagaaaaa 780
 tttggtggag atttgacagt taatagctat gaggagttag agagtttatt taaaaataag 840
 gaattgcac caatggattt aaaaaatgct gtagctgaag aacttataaa gatttttagag 900
 ccaattagaa agagatta 918

<210> 18
 <211> 306
 <212> PRT
 <213> artificial

<220>
 <223> tRNA synthetase based on M. jannaschii tyrosyl-tRNA synthetase

<400> 18

Met Asp Glu Phe Glu Met Ile Lys Arg Asn Thr Ser Glu Ile Ile Ser
 1 5 10 15

Glu Glu Glu Leu Arg Glu Val Leu Lys Lys Asp Glu Lys Ser Ala Leu
 20 25 30

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Ile Gly Phe Glu Pro Ser Gly Lys Ile His Leu Gly His Tyr Leu Gln
35 40 45

Ile Lys Lys Met Ile Asp Leu Gln Asn Ala Gly Phe Asp Ile Ile Ile
50 55 60

Leu Leu Ala Asp Leu His Ala Tyr Leu Asn Gln Lys Gly Glu Leu Asp
65 70 75 80

Glu Ile Arg Lys Ile Gly Asp Tyr Asn Lys Lys Val Phe Glu Ala Met
85 90 95

Gly Leu Lys Ala Lys Tyr Val Tyr Gly Ser Glu Phe Gln Leu Asp Lys
100 105 110

Asp Tyr Thr Leu Asn Val Tyr Arg Leu Ala Leu Lys Thr Thr Leu Lys
115 120 125

Arg Ala Arg Arg Ser Met Glu Leu Ile Ala Arg Glu Asp Glu Asn Pro
130 135 140

Lys Val Ala Glu Val Ile Tyr Pro Ile Met Gln Val Asn Gly Cys His
145 150 155 160

Tyr Arg Gly Val Asp Val Ala Val Gly Gly Met Glu Gln Arg Lys Ile
165 170 175

His Met Leu Ala Arg Glu Leu Leu Pro Lys Lys Val Val Cys Ile His
180 185 190

Asn Pro Val Leu Thr Gly Leu Asp Gly Glu Gly Lys Met Ser Ser Ser
195 200 205

Lys Gly Asn Phe Ile Ala Val Asp Asp Ser Pro Glu Glu Ile Arg Ala
210 215 220

Lys Ile Lys Lys Ala Tyr Cys Pro Ala Gly Val Val Glu Gly Asn Pro
225 230 235 240

Ile Met Glu Ile Ala Lys Tyr Phe Leu Glu Tyr Pro Leu Thr Ile Lys
245 250 255

Arg Pro Glu Lys Phe Gly Gly Asp Leu Thr Val Asn Ser Tyr Glu Glu
260 265 270

Leu Glu Ser Leu Phe Lys Asn Lys Glu Leu His Pro Met Asp Leu Lys
275 280 285

Asn Ala Val Ala Glu Glu Leu Ile Lys Ile Leu Glu Pro Ile Arg Lys
290 295 300

Arg Leu
305

<210> 19
<211> 306
<212> PRT
<213> artificial

<220>

<223> tRNA synthetase based on M. jannaschii tyrosyl-tRNA synthetase

<400> 19

Met Asp Glu Phe Glu Met Ile Lys Arg Asn Thr Ser Glu Ile Ile Ser
1 5 10 15

Glu Glu Glu Leu Arg Glu Val Leu Lys Lys Asp Glu Lys Ser Ala Leu
20 25 30

Ile Gly Phe Glu Pro Ser Gly Lys Ile His Leu Gly His Tyr Leu Gln
35 40 45

Ile Lys Lys Met Ile Asp Leu Gln Asn Ala Gly Phe Asp Ile Ile Ile
50 55 60

Leu Leu Ala Asp Leu His Ala Tyr Leu Asn Gln Lys Gly Glu Leu Asp
65 70 75 80

Glu Ile Arg Lys Ile Gly Asp Tyr Asn Lys Lys Val Phe Glu Ala Met
85 90 95

Gly Leu Lys Ala Lys Tyr Val Tyr Gly Ser Glu Phe Gln Leu Asp Lys
100 105 110

Asp Tyr Thr Leu Asn Val Tyr Arg Leu Ala Leu Lys Thr Thr Leu Lys
115 120 125

Arg Ala Arg Arg Ser Met Glu Leu Ile Ala Arg Glu Asp Glu Asn Pro
130 135 140

Lys Val Ala Glu Val Ile Tyr Pro Ile Met Gln Val Asn Gly Thr His
145 150 155 160

Tyr Arg Gly Val Asp Val Ala Val Gly Gly Met Glu Gln Arg Lys Ile
165 170 175

His Met Leu Ala Arg Glu Leu Leu Pro Lys Lys Val Val Cys Ile His
180 185 190

Asn Pro Val Leu Thr Gly Leu Asp Gly Glu Gly Lys Met Ser Ser Ser
195 200 205

Lys Gly Asn Phe Ile Ala Val Asp Asp Ser Pro Glu Glu Ile Arg Ala
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Lys Ile Lys Lys Ala Tyr Cys Pro Ala Gly Val Val Glu Gly Asn Pro
225 230 235 240

Ile Met Glu Ile Ala Lys Tyr Phe Leu Glu Tyr Pro Leu Thr Ile Lys
245 250 255

Arg Pro Glu Lys Phe Gly Gly Asp Leu Thr Val Asn Ser Tyr Glu Glu
260 265 270

Leu Glu Ser Leu Phe Lys Asn Lys Glu Leu His Pro Met Asp Leu Lys
275 280 285

Asn Ala Val Ala Glu Glu Leu Ile Lys Ile Leu Glu Pro Ile Arg Lys
290 295 300

Arg Leu
305

<210> 20
<211> 306
<212> PRT
<213> artificial

<220>
<223> tRNA synthetase based on M. jannaschii tyrosyl-tRNA synthetase
<400> 20

Met Asp Glu Phe Glu Met Ile Lys Arg Asn Thr Ser Glu Ile Ile Ser
1 5 10 15

Glu Glu Glu Leu Arg Glu Val Leu Lys Lys Asp Glu Lys Ser Ala Ala
20 25 30

Ile Gly Phe Glu Pro Ser Gly Lys Ile His Leu Gly His Tyr Leu Gln
35 40 45

Ile Lys Lys Met Ile Asp Leu Gln Asn Ala Gly Phe Asp Ile Ile Ile
50 55 60

Leu Leu Ala Asp Leu His Ala Tyr Leu Asn Gln Lys Gly Glu Leu Asp
65 70 75 80

Glu Ile Arg Lys Ile Gly Asp Tyr Asn Lys Lys Val Phe Glu Ala Met
85 90 95

Gly Leu Lys Ala Lys Tyr Val Tyr Gly Ser Glu Phe Gln Leu Asp Lys
100 105 110

Asp Tyr Thr Leu Asn Val Tyr Arg Leu Ala Leu Lys Thr Thr Leu Lys
115 120 125

Arg Ala Arg Arg Ser Met Glu Leu Ile Ala Arg Glu Asp Glu Asn Pro
130 135 140

Lys Val Ala Glu Val Ile Tyr Pro Ile Met Gln Val Asn Gly Gly His
145 150 155 160

Tyr Leu Gly Val Asp Val Ile Val Gly Gly Met Glu Gln Arg Lys Ile
165 170 175

His Met Leu Ala Arg Glu Leu Leu Pro Lys Lys Val Val Cys Ile His
180 185 190

Asn Pro Val Leu Thr Gly Leu Asp Gly Glu Gly Lys Met Ser Ser Ser
195 200 205

Lys Gly Asn Phe Ile Ala Val Asp Asp Ser Pro Glu Glu Ile Arg Ala
210 215 220

Lys Ile Lys Lys Ala Tyr Cys Pro Ala Gly Val Val Glu Gly Asn Pro
225 230 235 240

Ile Met Glu Ile Ala Lys Tyr Phe Leu Glu Tyr Pro Leu Thr Ile Lys
245 250 255

Arg Pro Glu Lys Phe Gly Gly Asp Leu Thr Val Asn Ser Tyr Glu Glu
260 265 270

Leu Glu Ser Leu Phe Lys Asn Lys Glu Leu His Pro Met Asp Leu Lys
275 280 285

Asn Ala Val Ala Glu Glu Leu Ile Lys Ile Leu Glu Pro Ile Arg Lys
290 295 300

Arg Leu
305

<210> 21
<211> 77
<212> RNA
<213> Methanococcus jannaschii

<400> 21
ccggcggguag uucagcaggg cagaacggcg gacucuaaa cgcgauggcg cugguucaaa
uccggcccgc cggacca

60

77

<210> 22
<211> 10
<212> PRT
<213> Artificial

<220>
<223> N-terminal tryptic peptide of Z domain protein including p-acetyl
-L-phenylalanine

<220>
<221> MISC_FEATURE
<222> (7)..(7)
<223> X is p-acetyl-L-phenylalanine

<400> 22

Met Thr Ser Val Asp Asn Xaa Ile Asn Lys
1 5 10

<210> 23
<211> 38
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<400> 23
gaggaatccc atatggacga atttgaaatg ataaagag

38

<210> 24
<211> 36
<212> DNA
<213> Artificial

<220>
<223> PCR primer

<400> 24
cgtttgaac tgcagttata atctctttct aattgg

36